Pestic. Sci. 1996, **48**, 199–204

Toxicity of Mixtures of *Bacillus thuringiensis* with Endosulfan and Other Insecticides to the Cotton Boll Worm *Helicoverpa armigera*

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(Received 20 October 1995; revised version received 20 February 1996; accepted 30 May 1996)

Abstract: In the laboratory, low concentrations of *Bacillus thuringiensis* Berliner (B.t.), as 'Dipel' 2X applied (at about the LC_5) to cotton leaves enhanced the activity of endosulfan and reduced resistance to endosulfan from approximately seven fold to two fold in neonate larvae of *Helicoverpa armigera* (Hubner). Low (LC_5) concentrations of endosulfan also increased the toxicity of B.t. Both endosulfan and B.t. increased the toxicity of the stomach poison cryolite to *H. armigera*. This increased toxicity was not observed where B.t./endosulfan combinations were applied topically to larvae or where B.t. was combined with organophosphorus or pyrethroid insecticides which are considered primarily to be contact toxicants. Mixtures of B.t. and endosulfan applied at equitoxic concentrations were less toxic than similar concentrations applied alone.

Key words: *Helicoverpa armigera*, *Bacillus thuringiensis*, resistance, insecticide mixtures, endosulfan

1 INTRODUCTION

Resistance to insecticides has been a recurrent problem in Australian populations of *Helicoverpa armigera* (Hubner).[1] A resistance management strategy, aimed primarily at resistance to pyrethroids and endosulfan, has been in widespread use since 1983. The strategy has successfully allowed continued use of these insecticides,[2] and the program has been an important factor in an expanding industry. Resistance to pyrethroids has been managed by a series of procedures involving restriction of pyrethroid use to specific stages of crop development, to use of refugia, and recently, to the addition of synergists. Resistance to endosulfan has been evident since the late 1960s and has been managed by restricted usages and influxes of susceptibles from refugia.

Conventional *Bacillus thuringiensis* Berliner (B.t.) alone has not been widely successful as a control for *Helicoverpa* spp. for a number of reasons including inadequate formulations, poor timing, application problems and lack of persistence.[3] In addition, costs of B.t. applications have not been competitive with those of contact insecticides. Recently, however, mixtures of B.t. and endosulfan or pyrethroids, often at reduced rates, have been used. These mixtures do not appear synergistic (Forrester N., 1994, pers. comm.) but their apparent usefulness warrants investigation. We have investigated the effects of B.t. on insect resistance to endosulfan and the potential for enhanced toxicity of mixtures of B.t. and various insecticides to *H. armigera*.

2 METHODS

2.1 Insect populations

Insects used were neonate larvae of *H. armigera* reared in the laboratory on artificial diet using methods described by Daly and Fisk.[4] Both the endosulfan-susceptible and endosulfan-resistant populations are described in Daly and Fisk[5] where they are given as suscept2 and resis2. Both populations are resistant to pyrethroids.

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Pestic. Sci. 0031-613X/96/$09.00

2.2 Bioassay methods

Endosulfan was used as a solution in acetone +0·5 g litre^{-1} 'Triton X' in distilled water (1 + 4 by volume). These solutions were prepared fresh weekly from a stock solution in acetone, and were used as prepared or held frozen at −12°C.

Suspensions of *B. thuringiensis* ('Dipel' 2X, Abbott Labs, Sydney; lot 22466-210C, dated 18 June, 1992) were prepared fresh daily from aliquots of 100 g litre^{-1} held frozen at −50°C. The activity of this sample was calibrated against a standard containing 50% of the international standard mg^{-1} (9350 IU mg^{-1} of *B.t. kurstaki* versus 18 700 IU mg^{-1} in the original standard). Calibration was from a concentration: response regression obtained with five concentrations of the standard incorporated into the artificial diet. Mortality of neonate larvae (*c.*100 per concentration) was assessed after five days. The B.t. 2X formulation was listed on the label as 32 000 IU but calibrated as *c.*40 000 IU mg^{-1}.

Fenvalerate (technical grade, Shell Australia, Melbourne) stock solutions were prepared in acetone and diluted in acetone + aqueous 'Triton X' as described for endosulfan.

Chlorpyriphos methyl 500 g litre^{-1} EC ('Reldan' 50 EC Roussel UCLAF, Concord, NSW), was diluted with distilled water.

Cryolite (Kryocide, Pennwalt Corp., Philadelphia, PA), supplied as 96% sodium fluoaluminate, samples were weighed and diluted to appropriate concentrations with distilled water. Suspensions were prepared fresh daily and stirred constantly during use.

Leaf discs (45 mm) were taken from second to fifth internode of cotton (*Gossypium hirsutum* L. cv. Deltapine 90) and treated with 0·5 ml of test suspension, using a Potter tower. Where mixtures were tested, endosulfan, chlorpyriphos, fenvalerate or cryolite treatments were applied first, allowed to air dry and B.t. suspensions applied over the dried deposits. Treated leaf discs were allowed to air dry, placed on a Whatman #1 filter paper (4·25 cm) on the lid of a plastic souffle (1 oz plastic souffle lid, SOLO Plastic Souffles, Code PL1 for top and P1000 for souffle, Solo Cup Co., Chicago, IL) and five neonate larvae placed on the upper surface of the disc. The lid and container were snapped together with the treated surface upwards and held for 48 h at 65% RH and 25°C, 14 : 10 h light : dark. Larvae incapable of coordinated movement were considered dead.

Initial assays of mixtures were with concentrations of each compound that individually caused (as calculated from logit regressions), 5, 10, 20, 30, 40, 50 and 60% mortality. Later tests were with either B.t. or endosulfan concentrations fixed at the LC_5 (approx.) for the *Helicoverpa* strain involved, combined with increasing concentrations of the other component.

In topical assays with endosulfan or B.t. (or combinations thereof), larvae were immobilized for treatment by placement on filter papers held in Petri dishes on trays of ice[6] and sprayed as described with a Potter tower. After treatment, larvae were transferred onto cotton leaf discs and held in the plastic souffles as described above. Tests were replicated 12 times with five larvae per replicate.

2.2 Analysis of data

All mortality data were transformed to logits and concentrations applied transformed to logarithms prior to analysis. Regressions of data and comparisons of slopes and LC_{50} values were calculated using GLIM 3·77.[7] Comparisons of regressions were determined using Chi-square to assess differences in intercepts at the 95% confidence level. In other tests, larval mortality was transformed logarithmically (x + 1) before comparison of means with a t-test ($P = 0·1$).[8]

3 RESULTS

Resistance to endosulfan in the resistant population of *Helicoverpa* was estimated at about seven-fold at the LC_{50} (Table 1). Chi-square analysis indicated that regressions were separate but parallel. This is slightly less than estimates for neonate larvae by Daly and Fisk[5] who indicated resistance levels at 14-fold. Results of tests with B.t. against these two populations indicated that, while the endosulfan-resistant population was slightly more sensitive, differences were not significant and slopes were not different (Table 1). When non-fixed rate mixtures of endosulfan and B.t. (where increasing concentrations of each were employed) were assayed against the susceptible population, a non-linear response was obtained (Fig. 1). Mixtures which caused mortalities up to *c.*50% appeared to enhance the toxicity of both endosulfan and B.t. but higher concentrations did not result in increased mortality (Fig. 1 a,b). Mortalities obtained were considerably less than additive for these products alone, suggesting interference at higher concentrations.

When concentrations of B.t. or endosulfan were fixed at the LC_5 level (approximately 100 mg litre^{-1} for B.t. and 10 mg litre^{-1} for endosulfan) and the concentration of the other product varied, toxicity of the mixture was generally enhanced (Table 1). Resistance in the endosulfan-resistant population was reduced to two-fold. However, for the endosulfan-susceptible population, differences in response between the endosulfan alone treatment and the B.t. low concentration: endosulfan combination were not significant. Differences between the resistant and susceptible populations treated with the B.t. fixed: endosulfan variable combination remained significant. Endosulfan at 10 mg litre^{-1} significantly enhanced the toxicity of B.t. to both

TABLE 1
Toxicity of Endosulfan and *Bacillus thuringiensis* (B.t.) to Two Populations of *Helicoverpa armigera*

Treatment	*Population*	LC_{50} (95% *CL*) (*mg litre*$^{-1}$)	*Slope* (±*SE*)	*Resistance level* [a]	
				Endosulfan	*B.t.*
Endosulfan	E-susceptible[b]	34·3 (28·3–41·6)	1·4 (±0·18)	—	
Endosulfan	E-resistant	235·6 (193·4–287·1)	1·4 (±16)	6·9	
B.t.	E-susceptible	2490 (1810–3440)	0·75 (±0·10	—	
B.t.	E-resistant	1740 (1320–2290)	0·85 (±0·11)	—	0·7
Endosulfan plus B.t. 100 mg litre^{-1}	E-susceptible	22·4 (17·3–29·0)	0·99 (±0·11)	0·65	
Endosulfan plus B.t. 100 mg litre^{-1}	E-resistant	68·7 (54·2–87·2)	1·23 (±0·12)	2·0	
B.t. plus Endosulfan 10 mg litre^{-1}	E-susceptible	280 (150–490)	0·41 (±0·07)	—	
B.t. plus Endosulfan 10 mg litre^{-1}	E-resistant	810 (580–1140)	0·68 (±0·09)	—	

[a] Resistance level calculated as: $\frac{LC_{50} \text{ test population}}{LC_{50} \text{ E-susceptible}}$.

[b] Endosulfan-susceptible.

endosulfan-resistant and -susceptible populations (Table 1), although differences between these populations remained.

The addition of low concentration of B.t. to fenvalerate or chlorpyriphos-methyl deposits did not result in enhanced toxicity (Table 2). However, addition of either endosulfan or B.t. after treatment of leaf discs with cryolite enhanced the toxicity of the cryolite. Combinations of endosulfan and B.t. applied topically to neonate larvae did not result in increased toxicity (Table 3). B.t. was almost non-toxic at the concentration tested (1000 mg litre^{-1}). This same concentration, applied to leaf surfaces was lethal to over 80% of larvae feeding on the treated surface. Endosulfan was also more toxic when applied to leaf surfaces than when applied topically to larvae.

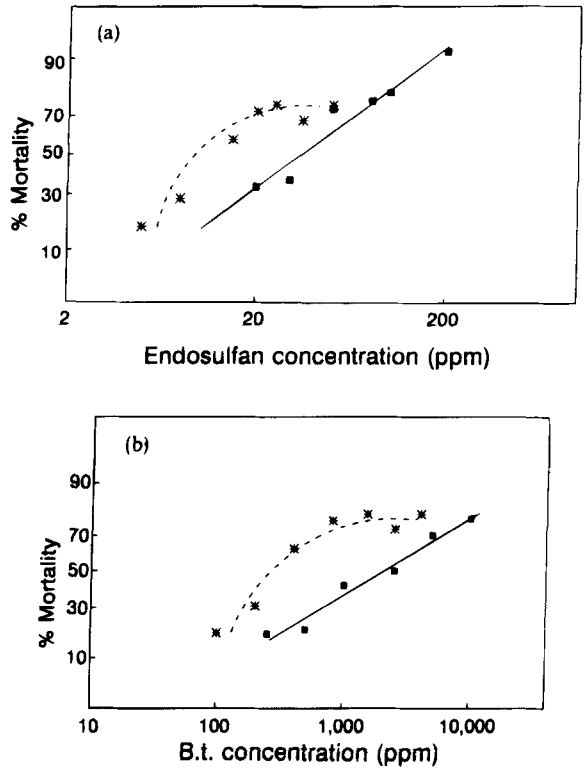

Fig. 1(a). Toxicity of endosulfan and endosulfan: *Bacillus thuringiensis* (B.t.) combinations to neonate larvae of *Helicoverpa armigera.* (■) Endosulfan alone, (*) endosulfan + B.t. (b). Toxicity of *Bacillus thuringiensis* (B.t) alone and in combination with endosulfan to neonate larvae of *Helicoverpa armigera.* (■) B.t. alone, (*) B.t. + endosulfan.

4 DISCUSSION

Mixtures of B.t. and endosulfan applied to cotton leaves became antagonistic at concentrations of B.t. or endosulfan that individually caused more than 60% mortality. However, inclusion of small amounts (at about the LC_5) of B.t. or endosulfan enhanced the toxicity of the other component of the mixture. Further, addition of small amounts of B.t. reduced resistance to endosulfan from about seven-fold to about two-fold. The increased toxicity of this mixture did not occur where applications were made topically to larvae or with mixtures where small amounts of B.t. were combined with an organophosphorus or pyrethroid insecticide. However, both B.t. and endosulfan increased the toxicity of the stomach poison, cryolite.

The observation that combinations of endosulfan and B.t. applied to cotton leaves were antagonistic at higher concentrations, at least against susceptible populations, suggests that application of mixtures of B.t. and endosulfan as recommended[9] might be less effective than each applied separately. However, these data are from

TABLE 2
Toxicity of Insecticides and Mixtures with *Bacillus thuringiensis* (B.t.) to *Helicoverpa armigera*

Treatment	*Concentration (mg litre^{-1})*	*Mortality (%)*	T *Value*	*Prob > /T/*[a]
Control	—	13·3		
B.t.	100	8·3		
Endosulfan	10	8·3	−3·58	0·0017
Endosulfan	10	35·0		
+ B.t.	100			
Endosulfan	50	73·3	−0·07	0·9486
Endosulfan	50	75·0		
+ B.t.	100			
B.t.	500	18·3	−3·82	0·0009*
B.t. +	500	51·7		
Endosulfan	10			
Cryolite	30 000	35·0	−1·98	0·0606*
Cryolite	30 000	61·7		
+ B.t.	100			
Cryolite	50 000	53·3	−2·29	0·322
Cryolite	50 000	71·7		
+ B.t.	100			
Cryolite	30 000	38·3	−1·73	0·0964*
Cryolite	30 000	56·7		
+ Endosulfan	10			
Cryolite	50 000	58·3	−2·02	0·0552*
Cryolite	50 000	81·7		
+ Endosulfan	10			
Fenvalerate	10	38·3	0·29	0·7756
Fenvalerate	10	33·3		
+ B.t.	100			
Fenvalerate	20	45·0	−0·62	0·7756
Fenvalerate	20	51·7		
+ B.t.	100			
Chlorpyriphos-methyl	10	18·3	−0·77	0·4488
Chlorpyriphos-methyl	10	16·7		
+ B.t.	100			
Chlorpyriphos-methyl	20	63·3	0·44	0·6648
Chlorpyriphos-methyl	20	58·3		
+ B.t.	100			

[a] Differences between compared treatments were considered significant when Prob > /T/ was <0·10; significant differences are indicated by an asterisk.

laboratory observations and differences in persistence of the B.t. and endosulfan residues may make the mixture useful under field conditions. Lack of enhanced toxicity with B.t./endosulfan mixtures has previously been observed by Forrester *et al.* (pers. comm.) but detrimental effects of the mixture as indicated here have not previously been reported. Whether the reduced toxicity of the mixtures at concentrations causing greater than *c.*60% mortality (for each component of the mixture) was related to reduced activity or reduced feeding by larvae was not readily apparent.

Low concentrations of B.t. reduced the level of resistance to endosulfan from about seven-fold to about two-fold. Low levels of resistance can be overcome by good application techniques and careful timing to impact the most sensitive life stages.[10,11] The low concentration of B.t. also affected the toxicity of endosulfan to the susceptible population (although to a lesser extent) suggesting that the mechanism involved or the system affected was not specific to the resistant population. The finding that low concentrations of endosulfan also enhanced the toxicity of B.t. might indicate a mechanism associated with uptake of both pesticides through the gut of *H. armigera* larvae. Enhanced toxicity was not observed when B.t./endosulfan mixtures were tested *via* topical application or where B.t. was combined with organophosphorus or pyrethroid insecticides which are considered primarily contact poisons. However, both

TABLE 3
Toxicity of Endosulfan : *Bacillus thuringiensis* Mixtures Applied Topically to Neonate Larvae of *Helicoverpa armigera*

Treatment	*Concentration (mg litre^{-1})*	*Mortality (%)*	T *Value*	*Prob > /T/*[a]
Control	—	9·2		
Endosulfan	100	25·0	−0·08	0·9395^{NS}
Endosulfan	100	30·0		
+ B.t.	1000			
Endosulfan	1000	95·0	1·69	0·1055^{NS}
Endosulfan	1000	76·7		
+ B.t.	1000			
B.t.	1000	11·7		

[a] Differences between compared treatments were considered significant when Prob > /T/ was <0·10.

B.t. and endosulfan enhanced the toxicity of cryolite, which is considered a stomach poison.[12] Endosulfan was *c.*10 times more toxic to larvae when applied to leaf discs compared to topical applications which also indicates that much of the toxicity of this compound to *H. armigera* larvae may be due to its activity as a stomach poison. Daly and Fisk[5], who exposed larvae of different instars to residues of endosulfan on cotton leaves, found that levels of resistance to endosulfan declined as larval size increased and increasing amounts of leaf area were consumed. The enhanced toxicity we observed might be described as synergism because the concentration of the second or added compound was at levels which added little direct toxicity. However, this is unlikely to be considered a truly synergistic mix because slopes of the dose: response regression were usually changed. There are several reports[13–16] which indicate enhanced or antagonistic effects of B.t. in combination with various insecticides. Few have related increased toxicity of mixtures to the activity of the components as stomach poisons, but recent studies[17,18] have shown that increased amounts of tannic acid in food resulted in an increased toxicity of B.t. to larvae of *Trichoplusia ni* (Hubner) and *Lymantria dispar* (L.). Low doses of B.t. mixed with various other chemicals have been successfully used to control the tobacco budworm (*Heliothis virescens* F.) in the US[19] but whether similar interactions to those reported here are responsible has not been determined.

There is considerable interest in increasing the use of B.t. on Australian cotton[3] but this has been hampered by high costs, lack of effectiveness, and lack of suitable materials for mixtures. The data presented here suggest that mixtures of B.t. and small quantities of endosulfan could result in increased effectiveness over B.t. alone and reduce resistance to endosulfan. These mixtures, while slightly more costly than B.t. alone, might result in increased use of this non-persistent, relatively selective product. Certainly, extensive field evaluation of this approach appears warranted.

ACKNOWLEDGEMENTS

We thank Karen Olsen, Anna Graboski (CSIRO) and Karen Whitty (AAFC) for technical assistance and Neil Forrester (NSW Agriculture) for his helpful suggestions on an earlier draft. R.T. Roush (Waite Institute, Adelaide) generously provided a sample of cryolite.

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